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Socioeconomic position during pregnancy and DNA methylation signatures at three stages across the early life: epigenome-wide association studies in the ALSPAC birth cohort

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Abstract

BACKGROUND

Socioeconomic experiences are recognized determinants of health, and recent work has shown that social disadvantages in early life may induce sustained biological changes at molecular level that are detectable later in life. However, the dynamics and persistence of biological embedding of socioeconomic position (SEP) remains vastly unexplored.

METHODS

Using the data from the ALSPAC birth cohort, we performed epigenome-wide association studies of DNA methylation changes at three life stages (birth, n=914; childhood at mean age 7.5, n =973; and adolescence at mean age 15.5, n=974), measured using the Illumina HumanMethylation450 Beadchip, in relation to pregnancy SEP indicators (maternal and paternal education and occupation).

RESULTS

Across the four early life SEP metrics investigated, only maternal education was associated with methylation levels at birth, and four CpGs mapped to *SULF1*, *GLB1L2* and *RPUSD1* genes were identified (FDR-corrected p-value < 0.05). No epigenetic signature was found associated with maternal education in children samples, while methylation levels at 20 CpG loci were found significantly associated with maternal education in adolescence. Although no overlap was found between the differentially methylated CpG sites at different ages, we identified two CpG sites at birth and during adolescence that are 219 bp apart in the *SULF1* gene that encodes an heparan sulfatase involved in modulation of signalling pathways. Using data from an independent birth cohort, the ENVIR*ON*AGE-cohort, we were not able to replicate these findings.

CONCLUSIONS

Taken together, our results suggest that parental SEP, and particularly maternal education may influence the offspring's methylome at birth and adolescence.

Key words

Social Class, DNA methylation, Occupations, Education

Key messages

- Recent evidence suggests that DNA methylation may play a key role in the embedding of SEP experiences during the life course.
- In this study we found that SEP has a modest influence on the methylome of the offspring at birth, with the strongest effects seen for maternal education.
- We have observed more differentially methylated CpG loci related to maternal education in adolescents than in newborns.
- We sought independent validation of the CpG sites found differentially methylated in relation to maternal education in cord blood, using neonatal biosamples from the ENVIR*ON*AGE study. Although one CpG site was found nominally significant, we did not consistently replicate the direction of this association.
- Although no overlap was found between the differentially methylated CpG sites at different ages, we identified two CpG sites at birth and during adolescence to be associated with SEP that are 219 bp apart in the *SULF1* gene that encodes an heparan sulfatase and is involved in modulation of signalling pathways.

Introduction

Individual chronic disease risk profiles in adulthood are not only driven by recent experiences (e.g. behaviours such as smoking and diet in adult life) but also, as formalised in the developmental origin of adult disease hypothesis, by combinations of *in utero* and early life exposures that influence health in a long-term fashion through processes known as biological embedding [1, 2]. Socioeconomic experiences are recognized determinants of health [3, 4], and recent work has shown that social disadvantages in early life may induce sustainable biological changes such as increased burden of inflammation [5, 6]. While evidence is accumulating to highlight the importance of the inflammatory response in the mediation of the SEP effect, a better understanding of the biological embedding may elucidate mechanisms that contribute to the early life influence of health inequalities [7]. DNA methylation may play a key role in the embedding of SEP experiences during the life course [8-10]. Several studies have investigated methylation changes associated with early life socioeconomic experiences in adults [11-20].

With few exceptions [14, 15, 17, 19], research found early life SEP associated with differential methylation in adulthood of gene promoters [11], repetitive elements [12], candidate genes involved in inflammatory and neuroendocrine responses [13, 16], and more recently with epigenetic age acceleration [18, 20].

In children evidence of an effect of early life SEP is still sparse [21-28]. Maternal education was found associated with placental hypomethylation of *HSD11B2*, which is involved in converting cortisol into inactive cortisone [21]; cord blood hypomethylation of imprinted genes [25]; and hypermethylation *INSIGF* and *LEP* genes, involved in growth and metabolism [22, 23], in children at the age of 17 months; while no effect on global methylation was detected both at birth and at three years [28]. Neighborhood-level poverty during pregnancy but not individual maternal education was found associated with (higher) methylation of repetitive elements in cord blood

[26], while another study found positive association with maternal education in only school boys [24]. Also, maternal socioeconomic position was associated in newborns with epigenetic acceleration [27].

Apart from being limited to candidate genes, a major limitation of previous research lays in study design. In practice, adult biosamples were retrospectively related to reported early life SEP [11-20], and biosamples collected at birth, childhood or adolescence were related to cross-sectional information on early life SEP [21-28]. By construction, these approaches didn't allow an appraisal of the temporal sequence of the events and might represent reverse causation due to the dynamic nature of epigenetic patterns [29]. The epigenome, in fact, varies over time as a function of environmental exposures, random processes and aging [30, 31]. Longitudinal studies based on repeated measures from the same individuals across life from birth onwards overcome these issues and may allow us to assess the temporal relationship between early life SEP and epigenetic changes [32].

In this context, we propose to use data from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, where methylation profiles are available at three time points in early life, to identify the early life SEP indicator mostly associated with epigenetic profiles at birth and to assess whether SEP-associated methylation changes at birth persist during childhood and adolescence.

Methods

Study population and methylation profiles

Our study population arises from the Accessible Resource for Integrated Epigenomics Studies (ARIES) project [33], a sub-study drawn from the ALSPAC mother-child cohort [34, 35] on a subset

of 1,018 mother-child pairs that has DNA methylation available. Ethical approval was obtained from the ALSPAC Ethics and Law Committee and the local research ethics committees, and mothers gave written informed consent. Characteristics of the ALSPAC and ARIES mother-child cohorts are summarized in the Table 1. A searchable data dictionary provides full information available on the ALSPAC study website http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/.

We analysed DNA methylation data of the offspring at the three time points (at birth, n=914; at mean age 7.5, n=973; and at mean age 15.5, n=974). A description of the data and sample collection and analyses of DNA methylation can be found in Supplementary Methods S1 pages 2-3.

Early life socioeconomic position indicators and covariates

Early life SEP was measured by parental education and occupation during pregnancy. Maternal and paternal education were collected from a self-reported questionnaire at 32 weeks of gestation and were coded in three categories according to educational achievement: (i) low: Certificate of Secondary Education (CSE), Vocational or Ordinary- (O-) level, educational qualifications generally obtained at 16 years of age; (ii) intermediate: Advanced- (A-) level, subject-specific qualifications most commonly attained at 18 years of age and required for admission to higher education; (iii) high: University degree and above.

Maternal occupation was collected from mothers' self-reported antenatal (18-week) questionnaire and paternal occupation from fathers' antenatal (32-week) questionnaire. Occupation was categorised according to the UK Registrar General's classification [36] and dichotomised into: (i) manual including unskilled, semi-skilled manual and skilled manual occupations; (ii) non-manual including skilled non-manual, managerial, technical and

professional occupations. Information on covariates collection can be found in Supplementary Methods S1 page 4.

Replication study

As an independent dataset from which to seek for validation, we used the ENVIRonmental influence ON AGEing (ENVIR*ON*AGE) birth-cohort [37]. Data and sample collection information and analyses of DNA methylation can be found in Supplementary Methods S1 pages 5-6.

Statistical analysis

Figure 1 depicts the study workflow which is structured in three phases:

(Figure 1 here)

i) Using the full resolution methylation data, we investigated the association between DNA methylation levels at birth and the four indicators of early life SEP: maternal and paternal education, and maternal and paternal occupation (Figure 1-A1). DNA methylation levels were modelled as dependent variable in a generalized linear model with beta-distributed response using the parameterization of Ferrari and Cribari-Neto [38] and we accounted for multiple testing by controlling the false discovery rate (FDR) [39] at a level below 0.05.

As a lower resolution alternative, we ran principal component (PC) analyses of the methylome using the prcomp function in R. We then regressed the PCs against each indicators of SEP (Figure 1-A2).

ii) For the followings two steps we selected one indicator of SEP based on its statistical significance in the PC analyses. We ran EWASs for the selected SEP indicator and DNA methylation status at childhood (Figure 1-B1) and adolescence (Figure 1-B2). Methylation levels of the probes significant in cord blood were integrated over the three time points (Figure 1-B3) according the method descripted in Supplementary Methods S1 pages 6.

iii) Finally, we adopted a targeted approach to seek independent validation of the CpG sites found differentially methylated in relation to the selected SEP indicator, using neonatal biosamples from the ENVIR*ON*AGE study (Figure 1-C).

All the analyses were adjusted for birth weight [40], parity [41], gestational age [40, 42] and sex of the newborn [43] in addition to technical variables: bead array row and bisulfite conversion batch.

To assess the robustness of our findings, we ran sensitivity analyses stratified by sex and including additional adjustment (i) on the possible explanatory variables of SEP: maternal age [44], BMI [40, 45], smoking status [46] and alcohol consumption during pregnancy [47], (ii) on blood cell composition which were estimated through an established deconvolution approach [48], (iii) on delivery mode and self-reported maternal health during the pregnancy, and (iv) for analyses at 7 and 15 years, on offspring life course characteristics: own BMI, own use of tobacco and alcohol (only for the analysis at 15 years).

To compare our results with previous targeted studies, we performed lookup analyses of methylation profiles at the three time points based on a list of 281 probes derived by CpG sites and genes previously associated with early life SEP [13, 16, 21-23, 25].

Results

Compared to the ALSPAC mothers, those included in ARIES were slightly older, more likely to have a higher educational level, non-manual occupations and being non-smokers during pregnancy.

In the ARIES subset smoking during pregnancy, higher BMI and younger age of the mothers at birth were more prevalent in lowest SEP group, and alcohol consumption was higher in the highest SEP group although not significantly (Table 1).

(Table 1 here)

These variables may act as mediators in the relationship between SEP and DNA methylation and were therefore excluded from the main analyses although were shown to affect cord blood DNA methylation (Supplementary Figure S2).

The SEP indicators were all significantly positively correlated with each other (r range=0.4-0.68) (Supplementary Figure S3).

Results of EWASs of DNA methylation in cord blood in relation to parental SEP indicators (maternal and paternal education and occupation) are reported in Figure 2.

(Figure 2 here)

Below the FDR level of 0.05 we identified (four) differentially methylated sites only in relation to maternal education (Table 2). The regression coefficients for these CpG sites for all the other SEP indicators are reported in Supplementary Table S4.

(Table 2 here)

EWASs using alternative early life SEP indicators yielded lower effect size estimates and weaker associations (Figures 2-B-D, for maternal occupation, and paternal education and occupations, Supplementary Figures S5-A-B for household highest education and occupation,

and Supplementary Figure S5-C for alternative coding of the occupations) than the analysis of maternal education. Additional adjustment of the full resolution analyses of the four indicators of SEP for possible explanatory variables, including maternal age, maternal BMI before the pregnancy, maternal smoking and alcohol consumption during pregnancy, did not yield additional associations except for three probes in relation to paternal occupation (Supplementary Figure S6).

Among the four probes significantly associated with maternal education only two sites (*cg02283643*, β=0.075, p-value=4.67e-8, q-value=0.011; *cg11489090*, β=-0.160; p-value=6.20e-7, q-value=0.036) remained statistically significant upon adjustment for maternal age and BMI, smoking status and alcohol consumption during pregnancy (*cg02283643*, β=0.082, p-value=4.91e-08, q-value=0.016; *cg11489090*, β=-0.179, p-value=7.29e-7, q-value=0.049) (Supplementary Figure S7). None of the four probe have been previously reported to be associated with maternal age [44], BMI [49], smoking [50] or alcohol consumption [51] during pregnancy by larger studies, including Pregnancy and Childhood epigenetics consortium. Albeit mitigated, consistent results were observed in both males and females for 3 CpG sites (*cg02283643, cg165894161 and cg11489090).* Only *cg07371530* had a much stronger association in females (β=0.40, p-value=1.33e-8) compared to males (β=0.06, p-value=0.43) and for this CpG site interaction between sex and maternal education (p-value for interaction= 0.01) was identified (Supplementary Table S8).

Figure 3-A shows that a considerable number (n=27) of the 100 strongest associations found with maternal education (x-axis) consistently ranked high (within the first percentile) in the analysis of paternal education. Paternal education showed a similar behaviour (Figure 3-B), while maternal or paternal occupation did seem to yield inconsistent ranking. Correlation between the strongest association from the analyses of maternal and paternal education in cord blood are reported in the Figure 3-C.

(Figure 3 here)

To capture the SEP influence on the overall methylome, we ran principal component (PC) analyses of the methylome as a lower resolution alternative to our full-resolution analyses. Regressing the PCs against the four early life SEPs under investigation, education of the mother was found significantly associated to the scores of the first PC, which explained 12.44% of the variability of cord blood DNA methylation, while none of the other components yielded significant associations (Figure 4 shows the first 5 components that explain 22% of the variance).

(Figure 4 here)

We did not identify any differentially methylated sites in relation to the education of the mother in seven-year-olds but found 20 significant associations in adolescents (Table 2). No CpG site of this set of 20 CpG sites was significantly differentially methylated in either cord blood or childhood biosamples (Table 3). As for cord blood analysis, results were consistent in both males and females, although significance was weaker especially for males (Supplementary Table S9). Adjustment on child life course characteristics (BMI, smoking and alcohol consumption) did not affect direction and strength of associations although in general slightly increased the p-value (Supplementary Table S10).

(Table 3 here)

Also the CpGs identified in cord blood were not found significantly differentially methylated in either childhood or adolescent biosamples (Supplementary Table S11). Using a longitudinal model confirmed non–persistence of the neonatal epigenetic marks in later life time points (Supplementary Table S12).

Nevertheless, from our EWASs in cord and in adolescent blood, we identified differentially methylated CpG sites on the same gene: one site located in *SULF1* gene (*cg02283643,* located in the TSS200 region, p-value =4.67e-08) for cord blood samples, and another site for adolescents *(cg05806180,* located in the 5'UTR region, p-value=1.29e-06). Correlation of these sites was significant both in the analyses of cord (r=0.21, p-value=4.65e-10) and adolescent blood (r=0.17, p-value=4.80e-08) (Supplementary Figure S13). These two CpG sites are only 219 bp distant and show a similar magnitude and direction of methylation (*cg02283643,* β=0.07; *cg05806180,* β=0.10). The probe (*cg02283643*) located on *SULF* and found significant in cord blood is the only one to remain significant even after adjustment for delivery mode and maternal health during the pregnancy, and white blood cells composition (Supplementary Table S14).

We interrogated the methylation levels at the four CpG loci found differentially methylated in cord blood in relation to maternal education in the ENVIR*ON*AGE cohort and were not able to replicate the findings. Compared to results from ARIES same direction of association was detected for only one CpG *cg02283643* (ENVIR*ON*AGE, β=0.017; ARIES, β=0.075) (Table 2 and Table 4), however p-value was >0.05 (p-value=0.76).

(Table 4 here)

At the opposite, another CpG site (*cg07371530*) was found nominally significant (p-value<0.05) but the direction of association did not consistently replicate (ENVIR*ON*AGE, β=-0.047; ARIES, β=0.247) (Table 2 and Table 4). Also, none of the 20 CpG sites found significant in ARIES adolescents was replicated in ENVIRONAGE (Supplementary Table S15).

In the lookup analyses we did not identify any significant probe, however *BDNF* gene appeared to be the top hit in the analyses at all the three time points (Supplementary Figure S16).

Discussion

One of the main findings of our study was that the impact of maternal education may be embedded in the offspring's methylome.

Education attainment, occupation and income are valid indicators to define the SEP and social inequality [52]. As expected, the measures of SEP we used in our study were all significantly correlated to each other, however maternal education was less correlated with maternal occupation as compared to paternal education with occupation. This can be partly attributable to the fact that our classification of occupation in manual and non-manual according the UK Registrar General's classification was developed for male worker and may poorly apply to female.

Each indicator measures different, often related aspects of socioeconomic stratification and may be more or less relevant to different health outcomes at different stages in the life course [53].

Occupational levels reflect access to material resources, prestige and exposure to occupational toxicants or physical workload [52]. Specifically for infants, maternal employment reflects prestige, access to material resources and it has been associated with better pregnancy outcomes [54]. However specific maternal occupations, such as those involving exposure to endocrine disruptors [55] or heavy physical work [56], may directly affect pregnancy outcomes, although effect sizes are generally small [57]. Intuitively maternal occupation has a larger effect on birth outcomes than paternal occupation, especially when considering occupation with specific toxic risks [58], while the contrary seems to happen later in life [59], because prestige and access to resource became more influential. Despite this, in our study we weren't able to detect any epigenetic signal in relation to maternal or paternal occupation. A possible explanation could be that we used a broad classification of occupation in manual and non-manual class that may have led to

misclassification of occupational exposures. Similarly, previous studies in ALSPAC cohort failed to detect adverse pregnancy outcomes in relation to maternal [60] or paternal occupation [61].

The level of education has been postulated as the dimension of the SEP that most strongly and consistently predicts health, especially for women and their children [53, 62, 63]. In support of these observations, we found an epigenetic link between education and the methylome. A lower level of education might affect birth outcomes directly limiting the capacity to integrate within society and increasing the risk of poverty, or indirectly through maternal health behaviours [64]. The knowledge and skills achieved through education may affect a person's cognitive functioning, making them more amenable to health information messages, or able to access appropriate health services, which might be advantageous for the offspring. For example, before the pregnancy adverse birth effects can be mediated by unhealthy life style such as maternal smoking, alcohol consumption, malnutrition and stress. In this regard, a recent EWAS meta-analysis found overlaps between the epigenetic signals associated with education attainment and those previously described to be associated with own or prenatal smoking, suggesting that the associations with education attainment could be due to correlation with smoking [65]. After the birth, maternal behaviour in childcare may mediate negative effect on health outcomes in infants and child. For example, mothers with lower level of education are less likely to be aware of the benefits of maternal milk for very preterm infants [66], or to provide child immunization [67].

We found that maternal education was the most important SEP variable significantly affecting the offspring's methylome, both considering CpG loci (Figure 2) or principal components analyses of cord blood DNA methylation (Figure 4). These results suggest that the association of maternal SEP with offspring methylation at birth are likely to be driven via *in utero* mechanisms. The epigenome is thought to be particularly vulnerable to environmental factors

during embryogenesis and there is increasing evidence for a developmental plasticity in response to toxicological, hormonal, nutritional, social, and broad ecological environmental exposures [68]. A wealth of epidemiological data supports the associations between maternal BMI or malnutrition and smoking with intrauterine growth retardation and birth weight [69-71]. Studies on the ARIES cohort, here also under study, have found that maternal obesity and underweight as well as smoking affects the neonatal epigenome [49, 50, 72].

We found more robust effects in females than males. Similarly, a study in literature found SES risk in childhood more robustly associated with methylation in young adult female than in male [74], although in placenta samples the opposite trend has been described [21].

Whilst we have identified CpG sites differentially methylated in cord blood associated with maternal education, we did not observe persistence of these methylation differences in later time points suggesting that these associations fade during the first years of life. These specific epigenetic signals at birth might have downstream effects in early life rather than be persistent across the life course, yet this does not exclude the involvement of epigenetic mechanisms. Studies on the variation of methylation markers in the population and their stability over time are limited, especially in early life [31, 75-79]. Previous studies demonstrated intra-individual variability of methylome during the first two years of life is mainly located within genes with important biological functions including immunity and inflammation [31]. These results have been confirmed in a study within the first five years after birth [80]. In a different study based on the ARIES cohort there was also little evidence of an association between methylation during childhood or in adolescence and either of birth weight or gestational age, the authors speculated correspondingly that there appears to be a phase of rapid “catch-up” in methylation differences [81]. Similarly, non-persistence of associations over time is acknowledged as one possible reason of the lack of association of early life SEP with methylation acceleration in adulthood found in

ALSPAC mothers [17]. Besides, in the life course perspective it is possible that the time span considered in this study is too short to identify biological changes that become evident only in adulthood and older ages according to duration and intensity of exposure to the favourable or unfavourable SEP exposures throughout life [82].

We have observed 20 significant differentially methylated CpG loci related to maternal education in adolescents, while only four CpGs in newborns. The maternal SEP might be associated with stronger effects on DNA methylation over time compared to only during the pregnancy, though additional research using early life SEP-trajectories are warranted to explore these observations. In fact, we cannot exclude that these effects are associated with adolescent SEP, that in turn is related to childhood SEP. In this regard, adjustment for adolescent BMI, alcohol and tobacco consumption, that are associated with own SEP, lowered the significance of the epigenetic associations although didn't affect direction and effect sizes.

Of particular interest were two loci in the *SULF1* gene which were significantly associated with maternal education in either cord blood or during adolescence, and which were only 219 bp distant from each other. *SULF1* encodes an extracellular heparan sulfate endosulfatase which catalyzes the 6-O-desulfation of heparan sulfate proteoglycans co-receptors for heparin-binding growth factors and cytokine signalling pathways, and therefore has an important role in many biological processes, such as embryogenesis, cell signalling, angiogenesis and tumorigenesis [83-85]. In experimental studies the *SULF1* gene has been found hypermethylated in cancers, while in humans it was differentially methylated in essential hypertension cases in young adults [86]. We could also not replicate the CpG located on *SULF1* and the other three CpG loci in the ENVIR*ON*AGE birth cohort. In this regard, it might be spurious to generalize the maternal education of the two cohorts because there are more than 20 years between their sampling, public health information might evolve over time and the cohorts are in two different countries.

Although both cohorts are representative for their respective areas, the participants are on average somewhat more highly educated than the geographical area they represent. For example, the ALSPAC population has a shortfall in less affluent families compared with the Avon area, and those in ARIES were more highly educated compared with those not in ARIES [33, 34]. In this regard, ARIES sub-sample has been reported to be reasonably representative of the main study population[33] however we cannot exclude a bias in the selection which in turn could be related to different parameters[87]. In this study, which fits in a discovery framework, we are focussing on potential methylation targets and the reliability of our targets we identified should be further assessed by other population studies. Further, since the epigenome is under both genetic and environmental influences, the epigenetics response to an exposure can be variable between individuals, populations, over time and so forth. Mechanistic pathways through which parental SEP (behavioural, occupational exposures, psychosocial stress) can affect the offspring CpG methylation may differ between the two cohorts. Nevertheless heterogeneous methylation pattern can have similar phenotypic consequences over the life course [88].

Findings from this study should be interpreted with caution due to certain limitations. DNA methylation has been measured in peripheral blood cells and not in specific tissues; although tissue specificity is a well-established attribute of DNA methylation, there is no clear consensus on which tissue might be most relevant to study when considering the impact of SEP [30]. SEP embedding involves several processes [15, 89], hence DNA methylation of brain or immune cells could potentially provide more insight. Moreover, in a mixed cell population such as (cord) blood, cells may demonstrate similar phenotypes but with distinct methylation patterns [90] and SEP-linked differences in B- to T-cell ratios might account for some of our observations [91]. We did additionally adjust the significant CpG sites for the estimated blood cell composition [48] and the magnitude of the associations remained. To our knowledge, this is the first study exploring

the relationship between early life SEP and epigenome-wide DNA methylation at birth and subsequently during childhood.

Conclusion

Understanding the differences in methylation patterns across ages and the consistency across independent studies could be the key to interpret the biological pathways through which the socioeconomic environment relates to molecular changes in the body. Taken together, our study provides some evidence that parental SEP has a modest influence on the methylome of the offspring early in life, with the strongest effects seen for maternal education on the offspring's methylome at birth and adolescence.

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